

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

1. A gene expression and delivery system comprising:

- 5 (a) a replicon of flavivirus origin, which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle; and
- 10 (b) at least a second vector that is capable of expressing flavivirus structural protein(s) and/or any other proteins required for packaging of the replicon into infectious flavivirus-like particles which vector is engineered to prevent recombination with the replicon when in its presence.

2. A gene expression and delivery system according to claim 1 wherein the replicon of flavivirus origin includes the nucleotide sequence for a flavivirus 5' untranslated region (UTR), at least a portion of the 5' coding region for flavivirus core protein, the nucleotide sequence coding for the flavivirus nonstructural proteins, and part or all of the 3'-terminal sequence of a flavivirus 3'UTR, required for self-replication of flavivirus genomic material, which vector is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.

3. A gene expression and delivery system according to claim 1 wherein the replicon contains a sufficient amount of flavivirus 5' UTR and a sufficient amount of 5' flavivirus coding region for core protein required for RNA replication.

4. A gene expression and delivery system according to claim 1 wherein the replicon contains a flavivirus 5' UTR and at least about between 60 and 80 nucleotides from the 5' coding region for flavivirus core protein.

25 5. A gene expression and delivery system according to claim 1 wherein the replicon is derived from Kunjin virus and contains the Kunjin virus 5' UTR and at least 60 nucleotides of the Kunjin virus 5' core protein coding region.

6. A gene expression and delivery system comprising:

- 30 (a) a self-replicating expression vector of flavivirus origin which includes the nucleotide sequence for the flavivirus 5'UTR, at least a portion of the 5' nucleotide coding region for flavivirus core protein, the nucleotide coding region

for flavivirus non-structural proteins, a sufficient amount of the 3'-terminal region of the flavivirus 3'UTR required for self-replication of flavivirus genomic material wherein (i) the vector is adapted to receive at least a nucleotide sequence without disrupting the replication capabilities of the vector, (ii) the nucleotide sequence is inserted into the vector in a manner which deactivates expression of at least a gene that would otherwise code for a flavivirus structural protein and (iii) the inserted nucleotide sequence does not encode for the structural protein sequence that it deactivates; and

(b) at least a second vector that is (i) capable of expressing the flavivirus structural protein(s) that is not expressed by the self-replicating expression vector described in (a) and (ii) engineered to prevent recombination with the self-replicating vector described in (a) when in its presence.

7. A gene expression and delivery system according to claim 1 wherein the replicon is adapted to receive at least a nucleotide sequence at any point in the replicon that does not effect processing of flavivirus proteins and RNA replication.

8. A gene expression and delivery system according to claim 7 wherein the nucleotide sequence is inserted into the 3' UTR of the replicon.

9. A gene expression and delivery system according to claim 8 wherein the nucleotide sequence that is inserted into the 3' UTR of the replicon is preceded by an IRES sequence.

10. A gene expression and delivery system according to claim 6 wherein the nucleotide sequence is inserted within a structural gene.

11. A gene expression and delivery system according to claim 6 wherein the nucleotide sequence is inserted within the locality of at least a deleted gene encoding a structural protein.

12. A gene expression and delivery system according to claim 11 wherein the nucleotide sequence that is inserted possesses at its 3' end a termination codon and an IRES sequence.

13. A gene expression and delivery system according to claim 11 wherein the inserted nucleotide sequence possesses at its 3' end a 2A autoprotease sequence of foot and mouth disease virus.

14. A gene expression and delivery system according to claim 13 wherein the inserted nucleotide sequence possesses at its 5' end a mouse ubiquitin sequence.

5 15. A gene expression and delivery system according to claim 1 wherein the replicon includes after the 3' terminal sequence of a flavivirus 3'UTR a sequence cassette containing antigenomic ribozyme of the hepatitis delta virus and SV 40 polyadenylation signal, required for production of vector RNA with precise 3' terminus possessing high replication efficiency.

16. A gene expression and delivery system according to claim 1 wherein the replicon is an RNA based vector.

10 17. A gene expression and delivery system according to claim 16 wherein the replicon is an RNA based vector, which is capable of producing replicon RNA in *in vitro* transcription reactions by bacteriophage DNA-dependent RNA polymerases from plasmid DNAs incorporating corresponding bacteriophage promoters preceding the replicon sequence.

15 18. A gene expression and delivery system according to claim 1 wherein the replicon is a DNA based vector.

19. A gene expression and delivery system according to claim 1 wherein the replicon is a DNA based vector, which is capable of producing replicon RNA in cells by cellular DNA-dependent RNA polymerase from plasmid DNA incorporating mammalian expression promoters preceding the replicon sequence.

20 20. A gene expression and delivery system according to claim 1 wherein the replicon is derived from a single flavivirus species.

21. A gene expression and delivery system according to claim 1 wherein the replicon is derived from Kunjin virus.

22. A gene expression system according to claim 21 wherein the replicon is derived from the FLSD or FLSDX clones as herein described.

23. A gene expression system according to claim 21 wherein the replicon is selected from one of the following vectors: C20rep; C20DXrep; C20DXrepNeo; C20DX2Arep; C20DX2ArepNeo; C20DX/CAT/2Arep; C20DX/CAT/2ArepNeo; C20DXIRESrep; C20DX/CAT/IRESrep; C20DX/GFP/2Arep; C20DX/GFP/2ArepNeo; C20DX/hcvCORE160/2Arep; C20DX/hcvCORE191/2Arep; C20DX/hcvNS3/2Arep;

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C20DX/VSV-G/2Arep; C20DX/B-GAL/2Arep; C20DXUb2A\_HDVrep or pKUNRep1, as described herein.

24. A gene expression and delivery system according to claim 1 wherein the second vector is heterologous in origin to the origin of the replicon.

25. A gene expression system according to claim 1 wherein the second vector is derived from an alphavirus.

26. A gene expression system according to claim 25 wherein the second vector is derived from Semliki Forest Virus.

27. A gene expression system according to claim 25 wherein the second vector is derived from Sindbis virus.

28. A gene expression system according to claim 24 wherein the second vector is derived from an adenovirus virus.

29. A gene expression system according to claim 24 wherein the second vector is derived from a fowlpox virus.

30. A gene expression system according to claim 24 wherein the second vector is derived from vaccinia virus.

31. A gene expression system according to claim 24 wherein the second vector is derived from baculovirus adapted for delivery of DNA expression cassette into mammalian cells.

32. A gene expression system according to claim 24 wherein the second vector is derived from plasmid DNA that allows transient, continuous or inducible expression of genes in mammalian cells.

33. A gene expression system according to claim 1 wherein the replicon is derived from Kunjin virus and the second vector is derived from Semliki Forest Virus.

34. A gene expression system according to claim 1 wherein the replicon is derived from Kunjin virus and the second vector is derived from Sindbis virus.

35. A gene expression system according to claim 1 wherein the replicon is adapted to include part or all of the following: at least, about the first 150 nucleotides of a flavivirus genome; at least about the last 60 nucleotides of E protein; substantially all of the nonstructural region; and part or all of the 3'UTR.

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36. A gene expression system according to claim 1 wherein the replicon is adapted to include part or all of the following: the first 157 nucleotides of the KUN genome, the last 66 nucleotides of E protein, the entire nonstructural region, and all of the 3'UTR.

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37. A gene expression system according to claim 1 wherein the replicon encodes all flavivirus structural proteins except for flavivirus core protein and the second vector is SFV-C as herein described.

38. A gene expression system according to claim 1 wherein the replicon encodes flavivirus core protein and the second vector is SFV-prME as herein described.

39. A gene expression system according to claim 1 wherein the replicon does not encode any flavivirus structural proteins and the second vector is SFV-prME-C and SFV-prME-C105 as herein described.

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40. A flavivirus replicon selected from the following: C20DX2Arep; C20OX2ArepNeo; C20DX/CAT/2Arep; C20DX/CAT/2ArepNeo; C20DXIRESrep; C20DX/CAT/IRESrep; C20DX/GFP/2Arep; C20DX/GFP/2ArepNeo; C20DX/hcvCORE160/2Arep; C20DX/hcvCORE191/2Arep; C20DX/hcvNS3/2Arep; C20DX/VSV-G/2Arep; C20DX/ $\beta$ -GAL/2Arep; C20DXUb2A\_HDVrep or pKUNRep1; as herein described.

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41. A stably transformed cell line that contains at least a replicon as claimed in claim 40.

42. A stably transformed cell line which cell line contains at least a replicon as claimed in claim 40 wherein the replicon includes either IRES-Neo or IRES-pac cassettes in the 3'UTR.

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43. A method for producing a stable cell line capable of persistently producing replicon RNA'S, comprising the steps of:

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(i) introducing into a cell a replicon of flavivirus origin which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle; and

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(ii) culturing that cell line under conditions which permit cell growth and replication.

44. A method for producing a flavivirus like particles comprising the steps of:

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(i) introducing into a cell a replicon of flavivirus origin which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and

which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle;

- 5 (ii) introducing into a replicon-containing cell a second vector that is capable of expressing flavivirus structural protein(s) and any/or other proteins required for packaging of the self-replicating expression vector into flavivirus viral particles which vector is engineered to prevent recombination with the self-replicating vector when in its presence; and
- (iii) harvesting virus like particles containing the replicon.

10 45. A flavivirus like particle produced according to the method of claim 44.

46. A flavivirus like particle according to claim 45 wherein said particle contains replicon that is derived from a DNA based replicon vector.

47. A flavivirus like particle according to claim 45 wherein said particle contains a replicon that is derived from an RNA based replicon vector.

15 48. A DNA based replicon vector of flavivirus origin, wherein the vector comprises: (a) a complementary DNA sequence of flavivirus origin that is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle; (b) a mammalian expression promoter; 5' to the complementary DNA sequence of flavivirus origin; and (c) at least a second nucleotide sequence capable of terminating transcription of replicon RNA with a precise 3' terminus; and wherein the promoter and the second nucleotide sequence are capable of promoting transcription and terminating same, of flavivirus RNA within the nucleus of a transfected cell.

20 49. A DNA based replicon vector according to claim 48 wherein the complementary DNA sequence of the nucleotide sequence includes a flavivirus 6' untranslated region (UTR), at least a portion of the 5' coding region for flavivirus core protein, the nucleotide sequence coding for the flavivirus non-structural proteins, and part or all of the 3'-terminal sequence of a flavivirus 3'UTR, required for self-replication of flavivirus genomic material, which vector is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.

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50. A DNA based replicon vector according to claim 48 wherein the eucaryotic expression promoter is a cytomegalovirus early enhancer promoter region.

51. A DNA based replicon vector according to claim 48 wherein the replicon includes at its 3' end an antigenomic ribozyme of hepatitis delta virus and simian virus 40 polyadenylation signal cassette, required for production of efficiently replicating replicon RNA with the precise 3' terminus.

52. A DNA based replicon vector according to claim 48 wherein the replicon contains a sufficient amount of the complementary DNA sequence of the flavivirus 5' UTR and the 5' flavivirus coding region for core protein required for RNA replication.

53. A DNA based replicon vector according to claim 48 wherein the replicon contains the complementary DNA sequence of a flavivirus 5' UTR and at least about between 60 and 80 nucleotides from the 5' coding region for flavivirus core protein.

54. A DNA based replicon vector according to claim 48 wherein the replicon is derived from Kunjin virus and contains the complementary DNA sequence of the Kunjin virus 6' UTR and at least 60 nucleotides of the Kunjin virus 5' core protein coding region.

55. A DNA based replicon vector according to claim 48 wherein the replicon includes the complementary DNA sequence of the nucleotide sequence for the flavivirus 5' UTR, at least a portion of the 5' nucleotide coding region for flavivirus core protein, the nucleotide coding region for flavivirus nonstructural proteins, a sufficient amount of the 3'-terminal region of the flavivirus 3' UTR required for self-replication of flavivirus genomic material wherein (i) the vector is adapted to receive at least a nucleotide sequence without disrupting the replication capabilities of the vector, (ii) the nucleotide sequence is inserted into the vector in a manner which deactivates expression of at least a gene that would otherwise code for a flavivirus structural protein and (iii) the inserted nucleotide sequence does not encode for the structural protein sequence that it deactivates.

56. A DNA based replicon vector according to claim 48 wherein the replicon is adapted to receive at least a nucleotide sequence at any point in the replicon that does not effect processing of flavivirus proteins and RNA replication.

57. A DNA based replicon vector according to claim 48 wherein the nucleotide sequence is inserted into the 3' UTR of the replicon.

58. A DNA based replicon vector according to claim 48 wherein the nucleotide sequence that is inserted into the 3' UTR of the replicon is preceded by an IRES sequence.

69. A DNA based replicon vector according to claim 48 wherein the nucleotide sequence is inserted within a structural gene.

60. A DNA based replicon vector according to claim 48 wherein the nucleotide sequence is inserted within the locality of at least a deleted structural gene.

61. A DNA based replicon vector according to claim 48 wherein the nucleotide sequence that is inserted in place of deleted structural proteins of the replicon is followed by a termination codon and a IRES sequence.

10 62. A DNA based replicon vector according to claim 48 wherein the inserted nucleotide sequence possesses at its 3' end a 2A autoprotease sequence of foot and mouth disease virus.

63. A DNA based replicon vector according to claim 48 wherein the inserted nucleotide sequence possesses at its 5' end a mouse ubiquitin sequence.

15 64. A DNA based replicon vector according to claim 48 wherein the replicon is derived from a single flavivirus species.

65. A DNA based replicon vector according to claim 48 wherein the replicon is derived from Kunjin virus.